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**Skriner**

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(54) **MARKERS FOR THE DIAGNOSIS OF CELIAC DISEASE**

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(52) **U.S. Cl.**

CPC ..... **C07K 14/00** (2013.01); **G01N 33/543** (2013.01); **G01N 33/564** (2013.01); **G01N 33/581** (2013.01); **G01N 33/68** (2013.01); **G01N 33/6854** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The invention relates to new peptides and to their use in the diagnosis of celiac disease.

**13 Claims, 4 Drawing Sheets**

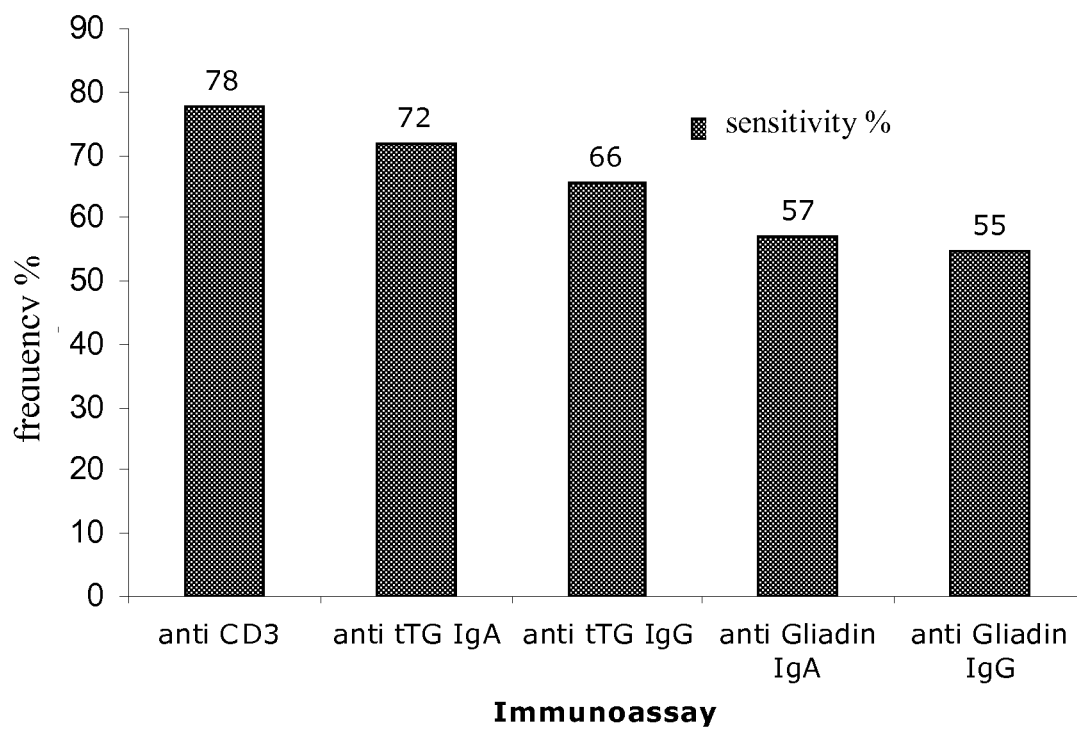


FIG. 1

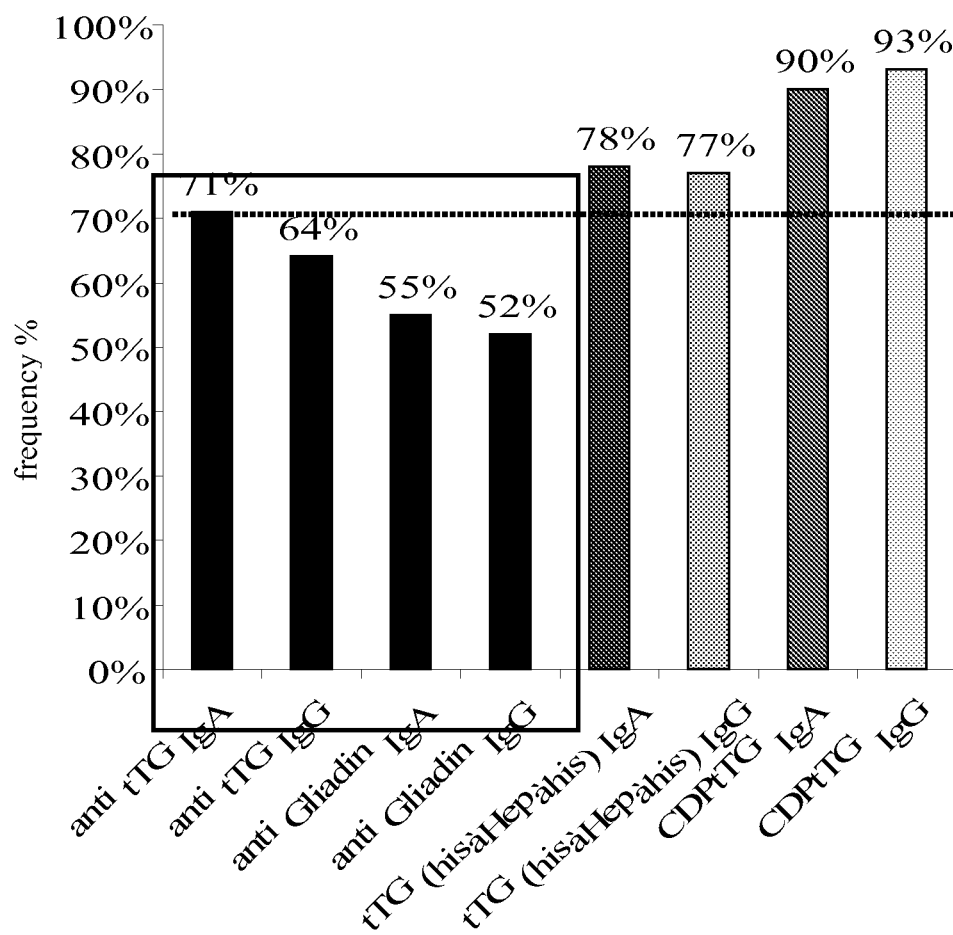


Fig. 2

negative characteristics	Number of negative sera	positive sera by assay [%]				
		tTG IgA	tTG IgG	Gliadin IgA	Gliadin IgG	CDP Peptid
tTG IgA negativ	26	(n.e.4)	<b>46</b>	15	<b>46</b>	<b>46</b>
tTG IgG negativ	31	<b>55</b>	(n.e.4)	39	29	52
Gliadin IgA negativ	38	42	50	(n.e.4)	42	<b>55</b>
Gliadin IgG negativ	41	66	49	46	(n.e.4)	<b>71</b>
CDP negativ	20	30	25	20	<b>40</b>	(n.e.4)
tTG IgA/ IgG negativ	14	(n.e.4)	(n.e.4)	21	<b>36</b>	29
Gliadin IgA/IgG negativ	22	55	41	(n.e.4)	(n.e.4)	<b>64</b>
tTG IgA; Gliadin IgA negativ	22	(n.e.4)	50	(n.e.4)	<b>55</b>	46
tTG IgA; Gliadin IgG negativ	14	(n.e.4)	50	29	(n.e.4)	50
tTG IgG; Gliadin IgA negativ	19	<b>42</b>	(n.e.4)	(n.e.4)	32	37
tTG IgG; Gliadin IgG negativ	22	<b>59</b>	(n.e.4)	45	(n.e.4)	50
tTG IgA/IgG; Gliadin IgA negativ	11	(n.e.4)	(n.e.4)	(n.e.4)	<b>45</b>	27
tTG IgA/IgG; Gliadin IgG negativ	9	(n.e.4)	(n.e.4)	<b>33</b>	(n.e.4)	<b>33</b>
Gliadin IgA/IgG; tTG IgA negativ	10	(n.e.4)	40	(n.e.4)	(n.e.4)	<b>50</b>
Gliadin IgA/IgG; tTG IgG negativ	12	<b>50</b>	(n.e.4)	(n.e.4)	(n.e.4)	42
tTG; Gliadin negativ	6	(n.e.4)	(n.e.4)	(n.e.4)	(n.e.4)	30

Fig. 3

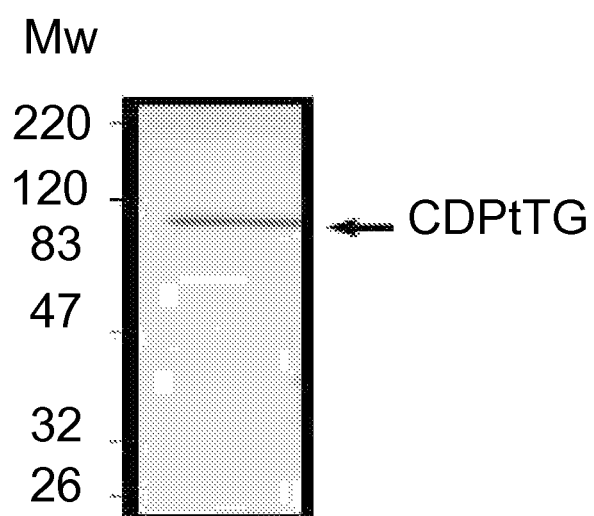


Fig. 4

1

**MARKERS FOR THE DIAGNOSIS OF CELIAC DISEASE****PRIORITY CLAIM**

This is a U.S. national stage of application No. PCT/EP10/064650, filed on Oct. 1, 2010. Priority is claimed on the following applications: German Application NO.: 10 2009 045 268.0 Filed on Oct. 1, 2009, the contents of which are incorporated here by reference.

**SEQUENCE LISTING**

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Apr. 26, 2012, is named 566129PU.txt and is 20,834 bytes in size.

**BACKGROUND OF THE INVENTION**

Celiac disease (according to ICD-10, WHO 2006 version: K90.0), also referred to as gluten-sensitive or gluten-induced enteropathy, intestinal infantilism; or non-tropical or endemic sprue, gluten intolerance or Heubner-Herter disease in adults, is a chronic disease of the small intestinal mucosa resulting from a hypersensitivity to gluten, a protein which is found in many types of grains. The intolerance remains throughout life and is in part genetically determined and cannot at present be treated causally.

Foods containing gluten give rise to inflammation of the small intestinal mucosa with frequently extensive destruction of the intestinal epithelial cells, so that nutrients are poorly absorbed and remain undigested in the bowels. Accordingly, the symptoms are weight loss, diarrhea, vomiting, anorexia, fatigue, ill-humor and, not least, failure to thrive during infancy. The severity of the condition can vary widely, making early diagnosis more difficult. Untreated celiac disease increases the risk of occurrence of non-Hodgkin lymphoma as well as carcinomas of the digestive tract, such as intestinal cancer. At present, the only treatment of celiac disease consists in gluten-free diet.

Meanwhile, a number of harmful peptide fragments of gluten have been identified. They all belong to the alcohol-soluble fraction (so-called prolamins) and are referred to as gliadin. In susceptible individuals, these peptide fragments give rise to a complex reaction of the intestinal mucosa and immune system. Mucosal cells of the small intestine produce increasing amounts of various classes of HLA (HLA-I, -DR and -DQ). Certain gliadin peptides bind to the HLA-DQ2 produced in increasing amounts. Said binding is increased as a result of glutamic acid formation from the amino acid glutamine which is present in the peptide in large numbers.

Formation of glutamic acid is mediated by the tissue transglutaminase enzyme, in particular tissue transglutaminase 2 (tTG2). As a result of this change, the corresponding section of gliadin has a better fit in the "pockets" of HLA proteins. The complex of gliadin peptide and HLA-DQ2 in turn binds to CD4+ T helper cells, causing increased production of various inflammatory mediators therein, for instance interferon- $\gamma$ , TNF $\alpha$ , interleukin-6 and interleukin-2. Various antibodies are formed during the further process of inflammation. In addition to antibodies against gliadin peptides themselves (gliadin antibodies, AGA), there are so-called autoantibodies against endogenous antigens. Tissue transglutaminase, particularly tTG2, has been identified as primarily responsible autoantigen. In view of these findings, celiac disease in patho-

2

physiological terms is understood to be a mixed form of allergy and autoimmune disease, wherein the allergic component in the form of hypersensitivity to the exogenous gliadin protein represents the precipitating factor, while the autoimmune response to endogenous structures is responsible for the severity of symptoms. Ultimately, the inflammatory process results in apoptosis of enterocytes, eventually leading to a more or less pronounced loss of small intestinal villi. As a result of the reduced absorption surface, the small intestinal mucosa damaged in this way is no longer capable of sufficiently transferring the supplied foods into the bloodstream.

In general, serological diagnostics of celiac disease involves testing for the presence of IgA and/or IgG type antibodies to gliadin or tTG2. One problem of well-known diagnostic markers is that the sensitivity of the tests is not yet optimal. Particularly the tests for the presence of gliadin antibodies exhibit low sensitivities of less than 80%. While well-known tests for tTG2 antibodies are more sensitive on the whole, the informative epitopes of tTG2 are so-called conformational epitopes, i.e. epitopes that can be recognized by antibodies only if tTG2 is presented in a non-denatured state. Consequently, tests for tTG2 antibodies are limited to those test methods wherein the tTG2 antigen is presented in a non-denatured state.

**SUMMARY OF THE INVENTION**

The object of the present invention is to alleviate or avoid one or more draw-backs of the prior art. More specifically, the object of the present invention is to provide new markers for the diagnosis of celiac disease.

Said object is accomplished by providing a peptide containing or consisting of an amino acid sequence of SEQ ID NO: 1. In a preferred fashion the peptide according to the invention may contain or consist of an amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

The present invention is based on the surprising finding that the peptide according to the invention is specifically recognized by antibodies produced in celiac patients. It was shown that the peptide of the invention can be used to identify even those celiac patients wherein recognition by means of one or more conventional serological celiac diagnostic tests has not been possible. Moreover, it was demonstrated that the peptide according to the invention is not a so-called conformational epitope but can also be recognized in denatured form by antibodies from patient sera. Consequently, the peptide is also suitable for types and variants of tests in which e.g. tTG2-based assays cannot be used as yet and wherein the epitope for antibody detection is used in denatured state, such as Western blot procedures. More specifically, it was shown that a peptide according to the invention, comprising a sequence of SEQ ID NOs: 1, 2, 3 and 4, and the sequence of human tTG2 can be used to provide a diagnostic assay for celiac disease which has a sensitivity that is higher than the sensitivity of any known diagnostic assay for celiac disease.

For the purpose of the present invention, the term "peptide" is understood to denote any molecule having a peptide bond between at least two amino acids. A peptide bond ( $-\text{NH}-\text{CO}-$ ) is an amide-type bond between the carboxyl group of a first amino acid and the amino group of a second amino acid. In principle, the term "peptide" therefore comprises dipeptides, oligopeptides, polypeptides and proteins, which peptides may have modifications.

The peptide according to the invention has at least one amino acid sequence of SEQ ID NO: 1 and thus at least one

3

amino acid sequence having 24 amino acids. Moreover, the peptide of the invention may comprise additional amino acids or amino acid sequences.

Amino acids are a class of organic compounds with at least one carboxyl group ( $-\text{COOH}$ ) and one amino group ( $-\text{NH}_2$ ). The amino acids present in the peptide according to the invention are preferably  $\alpha$ -,  $\beta$ - or  $\gamma$ -amino acids, more preferably  $\alpha$ -amino acids. Amino acids of the 20 naturally occurring amino acids, but also non-naturally occurring  $\alpha$ -,  $\beta$ - or  $\gamma$ -amino acids, can be included in the peptide.

One or more amino acids of the peptide according to the invention can be modified. A modified amino acid is understood to be an amino acid which bears a functional group on its side chain. The characteristic feature of a functional group is that the group imparts an additional function or property to the peptide, which is not or not sufficiently present in the peptide of the invention without the functional group, said functional group not being directly involved in specific binding of the peptide of the invention to antibodies from sera of celiac patients. Examples of functional groups are marker groups, such as GFP, His tag, AVI tag, biotin tag, etc., allowing, for example, detection, accumulation and/or purification of the peptide according to the invention. Other functional groups are coupling groups allowing e.g. reversible or irreversible coupling or immobilization of the inventive peptide to other molecules and/or carriers. With the aid of such coupling groups the peptide according to the invention can be, for example, bound to a molecule, such as BSA, or a microparticle, or can be immobilized on a carrier suitably prepared, if necessary. For example, a biotinylated peptide can be immobilized very effectively on a surface pre-treated with streptavidin (neutravidin). Examples of such coupling groups are biotin, streptavidin, etc., but it is also possible to use chemically reactive groups such as carboxyl, amino or amide groups. Suitable marker and/or coupling groups are well-known to those skilled in the art.

Apart from optional modifications present on the side chains of individual amino acids of the peptide according to the invention, the present invention also comprises peptides bearing modifications on their N- and/or C-terminal ends. In addition to the above-mentioned functional groups, the peptide may have functional groups on the N- and/or C-terminal ends which e.g. increase the stability of the peptide or facilitate the accessibility of the epitope included in the peptide according to the invention. Thus, for example, the peptide can be made more stable by coupling the peptide to antibodies.

The peptide according to the invention may have further amino acid sequences in addition to the amino acid sequences of SEQ ID NOs: 1, 2, 3 and/or 4. For example, the peptide may comprise a plurality of copies of a single sequence of SEQ ID NOs: 1, 2, 3 and 4.

The peptide according to the invention may have one or more amino acid sequences acting as linkers and/or functional groups. Thus, for example, two amino acid sequence regions of a peptide can be bound to each other by a linker. For example, an amino acid sequence which connects via peptide bonds two parts of the inventive peptide to be bound, so as to form a continuous amino acid chain, can be referred to as linker. Such linkers can also be referred to as peptide linkers. A part of the peptide according to the invention may also be in the form of a functional group. Such a functional group may involve the functional groups described above, attached on a side chain of one or more amino acids of the peptide according to the invention or situated at the N- and/or C-terminal ends of the peptide. Also, they can be in the form of an amino acid sequence and represent an integral component of the continuous amino acid chain of the peptide according to the

4

invention. Such peptidic functional groups may comprise amino acid chains having one or more amino acids or may comprise entire proteins or functional subunits of proteins. Examples of such functional groups are markers used in detection and purification, such as His tag, GFP, etc., or selected epitopes, such as portions or the entire sequence of human tTG2 (human tissue transglutaminase 2) of SEQ ID NO: 5.

More specifically, the peptide according to the invention may additionally comprise a sequence of at least 25 consecutive amino acids, preferably 100 consecutive amino acids, from the sequence of human tTG2 of SEQ ID NO: 5. In principle, the portions forming part of the peptide according to the invention can be selected from any part of the tTG2 sequence of SEQ ID NO: 5. In a preferred fashion the portion is selected such that it comprises at least one epitope that can be recognized by antibodies from sera of celiac patients. In a particularly preferred fashion the peptide of the invention comprises one or more partial sequences of tTG2 or one or more copies of the entire sequence of tTG2 of SEQ ID NO: 5 in addition to one or more amino acid sequences of SEQ ID NOs: 1, 2, 3 and/or 4. Moreover, the peptide of the invention may comprise additional amino acid sequences and/or functional groups, such as His tag, in particular a 6xHis tag (SEQ ID NO: 8). More specifically, the peptide of the invention may have or consist of a sequence of SEQ ID NO: 6 [SEQ ID NOs: 5+2] or SEQ ID NO: 7 [SEQ ID NOs: 5+2+2].

The present invention also relates to an inventive peptide for use as medicament, e.g. for use in diagnosis, especially in the diagnosis of celiac disease.

The peptide according to the invention can be prepared synthetically by controlled linking of selected amino acids. Alternatively, the peptide of the invention can be prepared by genetic engineering wherein a nucleic acid encoding the respective peptide is provided and placed in a context that allows expression and optionally subsequent purification of the encoded peptide. For example, expression can be effected in vitro or in transiently or stably transfected cells or in transformed microorganisms. Suitable methods are well-known to those skilled in the art and have been described e.g. in *Molecular Cloning—A Laboratory Manual*, 3<sup>rd</sup> Edition, Cold Spring Harbor Laboratory Press, 2001, or *Current Protocols in Molecular Biology*, John Wiley and Sons, NY (1989), and following issues.

The present invention also relates to a nucleic acid comprising a nucleic acid sequence encoding a peptide of the invention. A person skilled in the art will be aware that the genetic code is degenerate and, starting from a particular amino acid sequence of the peptide according to the invention, will be able to determine the possible nucleic acid sequences of an inventive nucleic acid without undue effort. Similarly, starting from a given nucleic acid sequence and taking into account the degeneration of the genetic code, a person skilled in the art can clearly and unambiguously determine without undue effort whether or not said given nucleic acid will encode a peptide according to the invention. The nucleic acid of the invention comprises DNA, RNA, mixtures and/or functional derivatives thereof, in particular cDNA, genomic DNA, linear or circular DNA, e.g. vectors, mRNA, linear or circular RNA, and can be prepared partially or completely by way of synthesis or genetic engineering. The nucleic acid can be in single-stranded form or partially or completely in the form of a double strand.

The nucleic acid according to the invention is preferably an isolated nucleic acid. For the purposes of the present invention, "isolated" means that the isolated component has been separated from its natural context. Using the example of an

5

isolated nucleic acid, an exemplary illustration of what the term "isolated" comprises at minimum will be given below. For example, a nucleic acid is isolated if it has been purposefully modified by man and/or if the nucleic acid has been transferred to an environment other than the natural environment or to a place other than the natural locus. Similarly, a nucleic acid is isolated in the meaning of the invention if it exists in a purified form or separated from its natural environment, preferably in a substantially pure and/or homogeneous form, or substantially free of nucleic acids which are not nucleic acids according to the invention. A cloned nucleic acid is generally an isolated nucleic acid.

The present invention also comprises transformed microorganisms or transformed cells comprising a nucleic acid according to the invention. This includes microorganisms and cells either transiently or stably transformed or transfected with a nucleic acid of the invention situated therein, in which event the nucleic acid of the invention may exist e.g. in free form in the microorganism or cell or incorporated in the genome. The microorganisms according to the invention are unicellular organisms, preferably bacteria or unicellular fungi such as yeasts. The cells according to the invention are cells of multicellular organisms, preferably isolated cells, e.g. cells allowing in vitro culturing in a cell culture. For example, the cells can be either primary cells or immortalized cells. In particular, those microorganisms or cells are preferred which can be used or have been used in the preparation of peptides and/or proteins.

The present invention also relates to isolated antibodies that bind to a peptide according to the invention. An antibody is understood to be a protein having one or more specific antigen binding sites (CDR, complementarity determining region). Antibodies in the meaning of the invention include both polyclonal or monoclonal antibodies having a classical antibody structure and derivatives or fragments derived therefrom, such as Fab, Fab<sub>2</sub>, single chains, etc. Starting from a particular peptide according to the invention, a person skilled in the art is able, without undue effort, to generate an isolated antibody that specifically binds to said peptide. Such techniques and approaches are well-known to those skilled in the art and routine in daily laboratory practice. For example, the isolated antibodies of the invention can also be generated in such a way that, by using a peptide according to the invention, the antibodies present in the serum of celiac patients are isolated, purified and thus made accessible. To this end, the peptide of the invention can be coupled to a support, for example, and the support loaded with peptide is subsequently contacted with celiac patient serum. Non-specifically bound components of the serum are removed, and the antibodies specifically bound to the peptide of the invention are subsequently eluted.

The antibodies according to the invention can be used, for example, for the detection of pathogens, e.g. peptidic pathogens, associated with celiac disease, preferably for the in vitro detection of such pathogens. In a preferred fashion such pathogens can be detected in foods in order to e.g. approve certain foods for celiac patients or delete them from a list of tolerable foods.

The invention also relates to a method for determining the safety of foods intended for consumption by celiac patients, which method is characterized in that the presence of celiac disease-associated pathogens in such foods is determined using the isolated antibodies according to the invention.

The present invention also relates to a method for the in vitro detection of antibodies against a peptide according to the invention in a sample. The method of the invention is characterized in that:

6

i) a peptide according to the invention is contacted with a sample in vitro, and

ii) antibody bound to the peptide is detected.

For the purposes of the present invention, the term "in vitro" is understood to denote any environment which is not inside a whole organism, e.g. a human or animal body.

An antibody is understood to be a protein having one or more specific antigen binding sites (CDR, complementarity determining region). Antibodies in the meaning of the invention include both polyclonal or monoclonal antibodies having a classical antibody structure and derivatives or fragments derived therefrom, such as Fab, Fab<sub>2</sub>, single chains, etc.

A sample is understood to be any composition to be investigated. The sample is preferably a biological or medical material, i.e., material obtained from an organism, parts of an organism, or from cells. Prior to use as sample in the method according to the invention, the material can be subjected to further treatment steps in order to e.g. condition the material so as to make it particularly suitable as sample in the method. More preferably, the sample is a material obtained from a body fluid or constituted of a body fluid. Preferred body fluids are blood, plasma, serum, synovial fluid, urine, stool, interstitial fluid, lymph, saliva, sweat, spinal fluid and/or lacrimal fluid. Particularly preferred are those body fluids wherein antibodies are present at high concentrations. Especially preferably, the body fluid is of human origin.

In the method according to the invention, a peptide of the invention is contacted in vitro with a sample to be investigated. The step of contacting is used to enable antibodies possibly included in the sample to bind to an epitope of the peptide according to the invention. To this end, the above step is carried out under conditions and in an environment allowing specific antigen-antibody binding. Suitable conditions are well-known to those skilled in the art. Such conditions preferably comprise a fluid environment and/or contacting at a temperature of from >0° C. to <60° C. Said contacting is preferably carried out for a period of time allowing formation of a specific antigen-antibody bond between the peptide of the invention and a peptide-specific antibody possibly included in the sample. The step of contacting is preferably performed for a period of more than 30 seconds, more preferably more than two minutes, and especially preferably for a period of from two minutes to 48 hours.

Antibody specifically bound to the peptide of the invention is detected in a sub-sequent step of the method according to the invention. For example, antibody specifically bound to the peptide of the invention can be detected in such a way that contacting is followed by removal of sample components not bound to the peptide of the invention, e.g. by means of one or more wash, purification or isolation steps, and subsequent use of agents allowing specific detection of antibodies. The above detection can be effected in one or more steps. For example, the agents used for the specific detection of antibodies can themselves be antibodies. Detection can be effected using e.g. a color reaction mediated or induced directly or indirectly by the agents for the detection of antibodies. For example, the antibodies for the detection of specific antibodies can be bound to functional groups or molecules (e.g. enzymes) capable of mediating or inducing a color reaction under specific conditions.

In the method according to the invention, the peptide of the invention can be immobilized on a support during one or more or all steps of the procedure. Immobilization is understood to be any coupling, binding or other association between the peptide of the invention and the support that prevents separate movement of peptide and support. For example, molecules and/or surfaces configured so as to allow reversible or irre-



versible binding of the peptide of the invention can be used as support. To this end, the support and/or the peptide of the invention may have functional groups which promote and/or permit binding between peptide and support. Molecules such as BSA, tTG, or surfaces such as presented by microparticles, nanoparticles or magnetic beads, or surfaces of selected membranes, polymers (e.g. polystyrene), or microtiter plates or test strips comprising such surfaces may be mentioned as exemplary supports. Suitable supports and possible ways of binding peptide and support are well-known to those skilled in the art.

More specifically, the method according to the invention can be performed in the form of an immunoassay procedure, and suitable immunoassay procedures have been described in David Wild (Ed.), *The Immunoassay Handbook*, 3<sup>rd</sup> Edition, Elsevier Science Publishing Company, Amsterdam, Boston, Oxford 2005.

In a preferred fashion the method according to the invention can be performed in the form of an ELISA procedure (ELISA: enzyme-linked immunosorbent assay), and suitable ELISA techniques have been described e.g. in Goldsby, R. A., Kindt, T. J., Osborne, B. A. & Kubly, J. *Enzyme-Linked Immunosorbent Assay*; in: *Immunology*, 5<sup>th</sup> ed., pp. 148-150. W. H. Freeman, New York, 2003. To this end, a sample can be contacted with a peptide of the invention immobilized on a support, unbound components are partially or substantially removed, if necessary, and an antibody coupled or coupleable to a functional group is subsequently used to detect a sample antibody bound to the peptide. As a rule, detection proceeds via a visually detectable reaction. For example, the antibody used in detection can be specific for antibodies of a particular organism or a particular origin and/or for a specific form of antibody, preferably for a particular isotype, e.g. IgA, IgM and/or IgG type antibodies, more preferably for human IgA, IgM and/or human IgG.

The method according to the invention can also be carried out in other assay formats, preferably e.g. as an RIA (radio-immunological assay) or as an immunoassay in a test strip format.

The present method is suitable for the detection of antibodies against a peptide according to the invention, particularly for the detection of IgA, IgM and/or IgG type antibodies, preferably for the detection of antibodies of human origin. The method according to the invention can be used in diagnosis, especially in serological diagnosis, preferably in the diagnosis of celiac disease.

The present invention also comprises a kit for carrying out the method according to the invention. To this end, the kit may include a peptide of the invention immobilized on a support. In addition, the kit may include instructions for using the kit and/or performing the inventive method by means of said kit. In a preferred fashion the kit is designed in the form of an ELISA, or in particular as a strip test. That is, the kit according to the invention comprises the peptide of the invention and optionally further components for carrying out the inventive method in a form suitable for performing the method according to the invention in an ELISA and/or strip test format. More specifically, the kit may comprise the inventive peptide coupled to a test strip. The kit according to the invention may optionally include additional components for carrying out the method of the invention. For example, such components may comprise reaction vessels, filters, solutions and/or other agents. In particular, the kit according to the invention may include agents for the detection of antibodies, preferably IgA, IgM and/or IgG type antibodies, more preferably for the detection of antibodies of human origin.

The kit according to the invention can be used for carrying out a method of the invention. More specifically, the kit according to the invention is suitable for use in diagnosis, preferably in serological diagnosis, and especially preferably in the diagnosis of celiac disease.

The kit according to the invention can be characterized in that the kit additionally includes agents for the detection of IgA, IgM and/or IgG type antibodies, preferably for the detection of human IgA, IgM and/or IgG type antibodies.

In particular, the kit according to the invention can be used in the diagnosis of celiac disease.

## BRIEF DESCRIPTION OF THE DRAWINGS

### Figures:

FIG. 1 shows the sensitivities of commercially available anti-gliadin IgA and IgG ELISAs as well as anti-tTG IgA and IgG ELISAs compared to a CD3 ELISA and indicates the % rate of correctly classified celiac patient sera as a measure for the sensitivity of each test.

FIG. 2 shows the sensitivities of commercially available anti-gliadin IgA and IgG ELISAs as well as anti-tTG IgA and IgG ELISAs compared to CDPtTG ELISA and tTG ELISA, using the CDPtTG ELISA protocol, and indicates the % rate of correctly classified celiac patient sera as a measure for the sensitivity of each test.

FIG. 3 shows sensitivity overlaps between the individual forms of ELISA used and indicates the percentage of celiac sera not recognized by the quoted tests that were tested positive with the respective assay.

FIG. 4 shows that CD3-specific antibodies isolated from patient sera can specifically recognize CDPtTG in a Western blot.

## DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS

### EXAMPLES

#### 1. Preparation and Purification of CD3, tTG and CDPtTG

CD3 of SEQ ID NO: 4 was prepared by synthesis and biotinylated at the C-terminal lysine residue.

Human tTG2 of SEQ ID NO: 5, hereinafter referred to as tTG, was cloned in a vector with removable 6xHis tag (SEQ ID NO: 8) for recombinant expression and expressed according to a standard protocol, isolated using NINTA column purification, and the 6xHis (SEQ ID NO: 8) was removed.

CDPtTG of SEQ ID NO: 6, hereinafter referred to as CDPtTG, was likewise cloned in a vector with C-terminal 6xHis tag (SEQ ID NO: 8) for recombinant expression and expressed according to a standard protocol, isolated using NINTA column purification, and the His tag was removed.

#### 2. ELISA for CD3, tTG (Homemade) and CDPtTG

To detect CD3-specific antibodies, a CD3 peptide ELISA was developed wherein biotinylated CD3 of SEQ ID NO: 4 was initially coupled on a neutravidin-coated microtiter plate, followed by the protocol below:  
Neutravidin-CD3 Peptide ELISA

The wells of the microtiter plate were initially blocked with PBS/5% MP buffer overnight. Thereafter, the biotinylated peptides were applied using 500 pmol/well in PBS buffer each time. After incubation for 2 h, the CD3/neutravidin-coated plates were washed 4 times with PBS/0.1% TWEEN® and incubated for 1 h with patient serum at a dilution of 1:800 in PBS/2% MP. Thereafter, washing was repeated 4 times, followed by application of peroxidase-conjugated second Ab at a dilution of 1:5000. Incubation was also for 1 hour. Finally,

washing was repeated 4 times, followed by application of the substrate. The reaction with development of a blue color was quenched after 5 min using 0.5 M sulfuric acid. The resulting yellow color was measured photometrically at a measurement wavelength of 450 nm versus a reference wavelength of 620 nm using an ELISA reader and visualized with the aid of the Magellan software. Each solution was applied using 100  $\mu$ l per well. Blocking and the single washing steps were carried out using 300  $\mu$ l per well each time. The microtiter plates with peptide, patient serum and 2<sup>nd</sup> Ab were incubated with agitation at RT.

#### CDtTG, tTG ELISA Protocol:

To detect tTG2- or CDPtTG2-specific antibodies, a specific ELISA was developed wherein recombinant tTG of SEQ ID NO: 5 and CDtTG of SEQ ID NO: 6 were initially coupled to a MAXISORB® microtiter plate (Nunc) and the following protocol was subsequently used:

- A) Coupling buffer: 100 mM Tris, 10 mM NaCl, pH 7.8
- B) Wash buffer: 50 mM Tris-HCl, 150 mM NaCl, 10 mM EDTA, 0.1% Tween 20, pH 7.4
- C) Saturation buffer: 50 mM Tris-HCl, 150 mM NaCl, 0.5% BSA, 3% sucrose, pH 7.4
- D) Serum dilution buffer: 50 mM Tris-HCl, 150 mM NaCl, 0.5% Tween 20, pH 7.4

#### Procedure:

Coating on MaxiSorb plates from Nunc: Coating quantity: CDPtTG and tTG were each used at a concentration of 0.5  $\mu$ g/well.

Coating volume: 100  $\mu$ l/well. All tTGs were suitably diluted in coupling buffer A. For coating, the plates were incubated at 40° C. overnight.

Blank and 2<sup>nd</sup> Ab controls were carried along in ELISA implementation. The OD values of blank and 2<sup>nd</sup> Ab controls were subtracted in ELISA evaluation.

After coating the plates were washed with 3×300  $\mu$ l/well wash buffer B. Each wash step corresponds to 600 rpm on the ELISA shaker for 3 min. Thereafter, the plates were blocked with 300  $\mu$ l/well saturation buffer for 2 h at RT. The sera were diluted 1:800 in serum dilution buffer D and 100  $\mu$ l/well directly placed on the plates after blocking. Incubation at RT with shaking; washing with 5×300  $\mu$ l/well wash buffer B.

#### 2<sup>nd</sup> Ab:

<hlgA>HRP from Dako was diluted 1:1500 in wash buffer B, and 100  $\mu$ l/well was used;

<hlgG>-HRP from Dako at a dilution of 1:5000 likewise in wash buffer B, and 100  $\mu$ l/well was used.

Incubation for 1 h at RT with shaking. Washing with 4×300  $\mu$ l/well wash buffer B. Allowing reaction with 100  $\mu$ l/well TMB substrate (SERAMUNBLUE® fast) for 5 min. Thereafter, quenching with 100  $\mu$ l/well quenching soln. (0.5 M H<sub>2</sub>SO<sub>4</sub>) and evaluation in ELISA reader at 450 nm.

#### 3. Cohort of Celiac Patients Sera

In the course of the present work, human patient sera from 91 patients with positive celiac diagnosis, varying age, sex and pathological characteristics were employed. All sera used were obtained from the Department of Clinical Rheumatology at the Charité (Berlin). In addition, 80 sera from normal donors were processed as control group and compared with the group of autoimmune diseases in this work.

#### 4. Comparison of Sensitivities Obtained for CD3 ELISA, tTG ELISA, CDtTG ELISA and Commercially Available Gliadin and tTG ELISAs

The sera specified under Section 3 were then used in the ELISA tests specified under Section 2. In parallel, the same sera were also examined with commercially available celiac ELISA tests which react to either gliadin-specific antibodies or antibodies specific to tTG2. To this end, commercial anti-

htTG IgA or IgG ELISAs from Generic Assays GmbH (Germany) and commercial anti-gliadin IgA and IgG ELISAs from Generic Assays GmbH (Germany) were used.

As shown in FIG. 1, the CD3-specific ELISA was found to be more sensitive in correct classification of celiac patient sera compared to the commercially available anti-tTG and anti-gliadin ELISAs.

As shown in FIG. 2, ELISAs based on recombinant tTG2 were likewise superior to the commercially available anti-tTG and anti-gliadin ELISAs with respect to sensitivity. The CDPtTG-based ELISA was by far the most sensitive ELISA for correct classification of celiac patient sera. Thus, in total, the presence of the peptide sequence of SEQ ID NO: 1 according to the invention results in a celiac ELISA with improved sensitivity compared to commercial ELISAs and an anti-tTG ELISA carried out under the same conditions as the CDPtTG ELISA.

Thus, it was shown that peptides comprising the peptide sequence of SEQ ID NO: 1 according to the invention are informative and can be used in the diagnosis of celiac disease. Also, it was shown that an ELISA based on a peptide comprising the peptide according to the invention of SEQ ID NO: 1 has improved sensitivity.

FIG. 3 illustrates overlaps in the sensitivities of the CD3 ELISA with the commercially available anti-gliadin and anti-tTG ELISAs. It was found that the CD3 ELISA can detect sera from celiac patients that were not recognized by one or more or even all of the commercially available ELISA tests used. Thus, the use of a celiac assay based on a peptide comprising the peptide sequence of SEQ ID NO: 1 provides a valuable contribution to complete serological detection and diagnosis of celiac disease patients.

#### 5. CD3-specific Antibodies from Patient Sera

##### Affinity Purification of CD3 Antibodies

For isolation and purification of the affinity-specific antibodies against the CD3 peptide from patient sera, the biotinylated variant of the CD3 peptide was coupled to avidin or neutravidin microtiter plates, followed by using a pool of 24 strongly positive celiac sera with minor nonspecific background binding for immune complex formation. Initially, the microtiter plate (MTP) was blocked with PBST/5% MP for 1 h at RT. This was followed by 3 washings with PBS wash buffer, and the wells of the 96-well plate was coated with 5000 pmol/well CD3 peptide, which corresponds to a 10-fold capacity per well. After incubation at RT for 1 hour, the plate was washed 3 times with wash buffer. Thereafter, the sera were applied to the streptavidin plate or NEUTRAVIDIN® plate, to which end each patient serum was initially diluted 1:100 in PBST/2% MP. Subsequently, the sera were pooled and placed in the wells using a volume of 100  $\mu$ l/well. This was followed by incubation for 1 hour at RT. Thereafter, the contents of all wells were removed and recombined and used in another purification cycle or as a control for the affinity-specific purification effect in another purification cycle with the negative control peptide, Bor21 in the present case. After removal of the sera the plate was washed 4 times with wash buffer. The wells were subsequently spiked with 150  $\mu$ l/well glycine elution buffer and incubated on the shaker for 10 min.

Finally, the elution buffer was resuspended several times and removed from the wells and immediately combined with 1:10 1 M Tris-HCl pH 8.0 buffer. This was immediately followed by rebuffing by ultracentrifugation in AMICON® FALCONS® with a membrane cutoff of 55 kDa, using 5× a 5-fold volume of PBS buffer as counterbuffer. As control of success the batch was tested in the CD3 ELISA with positive

## 11

and negative control sera. The quantities of antibodies thus obtained were stored at 4° C. in PBS and used in further immunological tests.

All wash steps were performed using a volume of 300 µl/well. Furthermore, all incubation steps were carried out at RT on the ELISA plate shaker at a rotational speed of 600 rpm.

As shown in FIG. 4, the CD3 peptide antibodies thus isolated react specifically with CDPtTG in a Western blot experi-

## 12

ment. From this it follows that the antigen in the CD3 peptide is not a conformational antigen, but can also be detected in denatured state by CD3 peptide antibodies isolated from patient sera. Consequently, peptides comprising the peptide according to the invention of SEQ ID NO: 1 are not only suitable for use in diagnostic tests focusing on antigens in native state, but also for test formats wherein the antigen is used in denatured state, such as Western blot assays, protein arrays, LUMINEX® bead arrays and/or protein chip assays.

## SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 8

<210> SEQ ID NO 1

<211> LENGTH: 24

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 1

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1 5 10 15

Glu Gln Pro Phe Pro Gln Pro Glu  
20

<210> SEQ ID NO 2

<211> LENGTH: 30

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 2

Gln Glu Gln Pro Phe Pro Gln Pro Glu Gln Pro Phe Gln Pro Glu Gln  
1 5 10 15

Pro Phe Gln Glu Gln Pro Phe Pro Gln Pro Glu Gln Pro Phe  
20 25 30

<210> SEQ ID NO 3

<211> LENGTH: 31

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 3

Gln Glu Gln Pro Phe Pro Gln Pro Glu Gln Pro Phe Gln Pro Glu Gln  
1 5 10 15

Pro Phe Gln Glu Gln Pro Phe Pro Gln Pro Glu Gln Pro Phe Lys  
20 25 30

<210> SEQ ID NO 4

<211> LENGTH: 31

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<220> FEATURE:

<221> NAME/KEY: MOD\_RES

<222> LOCATION: (31)..(31)

<223> OTHER INFORMATION: Lys is biotinylated

<400> SEQUENCE: 4

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Gln Glu Gln Pro Phe Pro Gln Pro Glu Gln Pro Phe Gln Pro Glu Gln  
 1 5 10 15  
 Pro Phe Gln Glu Gln Pro Phe Pro Gln Pro Glu Gln Pro Phe Lys  
 20 25 30  
  
 <210> SEQ ID NO 5  
 <211> LENGTH: 687  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens  
  
 <400> SEQUENCE: 5  
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 Asn Gly Arg Asp His His Thr Ala Asp Leu Cys Arg Glu Lys Leu Val  
 20 25 30  
 Val Arg Arg Gly Gln Pro Phe Trp Leu Thr Leu His Phe Glu Gly Arg  
 35 40 45  
 Asn Tyr Glu Ala Ser Val Asp Ser Leu Thr Phe Ser Val Val Thr Gly  
 50 55 60  
 Pro Ala Pro Ser Gln Glu Ala Gly Thr Lys Ala Arg Phe Pro Leu Arg  
 65 70 75 80  
 Asp Ala Val Glu Glu Gly Asp Trp Thr Ala Thr Val Val Asp Gln Gln  
 85 90 95  
 Asp Cys Thr Leu Ser Leu Gln Leu Thr Thr Pro Ala Asn Ala Pro Ile  
 100 105 110  
 Gly Leu Tyr Arg Leu Ser Leu Glu Ala Ser Thr Gly Tyr Gln Gly Ser  
 115 120 125  
 Ser Phe Val Leu Gly His Phe Ile Leu Leu Phe Asn Ala Trp Cys Pro  
 130 135 140  
 Ala Asp Ala Val Tyr Leu Asp Ser Glu Glu Glu Arg Gln Glu Tyr Val  
 145 150 155 160  
 Leu Thr Gln Gln Gly Phe Ile Tyr Gln Gly Ser Ala Lys Phe Ile Lys  
 165 170 175  
 Asn Ile Pro Trp Asn Phe Gly Gln Phe Glu Asp Gly Ile Leu Asp Ile  
 180 185 190  
 Cys Leu Ile Leu Leu Asp Val Asn Pro Lys Phe Leu Lys Asn Ala Gly  
 195 200 205  
 Arg Asp Cys Ser Arg Arg Ser Ser Pro Val Tyr Val Gly Arg Val Val  
 210 215 220  
 Ser Gly Met Val Asn Cys Asn Asp Asp Gln Gly Val Leu Leu Gly Arg  
 225 230 235 240  
 Trp Asp Asn Asn Tyr Gly Asp Gly Val Ser Pro Met Ser Trp Ile Gly  
 245 250 255  
 Ser Val Asp Ile Leu Arg Arg Trp Lys Asn His Gly Cys Gln Arg Val  
 260 265 270  
 Lys Tyr Gly Gln Cys Trp Val Phe Ala Ala Val Ala Cys Thr Val Leu  
 275 280 285  
 Arg Cys Leu Gly Ile Pro Thr Arg Val Val Thr Asn Tyr Asn Ser Ala  
 290 295 300  
 His Asp Gln Asn Ser Asn Leu Leu Ile Glu Tyr Phe Arg Asn Glu Phe  
 305 310 315 320  
 Gly Glu Ile Gln Gly Asp Lys Ser Glu Met Ile Trp Asn Phe His Cys  
 325 330 335  
 Trp Val Glu Ser Trp Met Thr Arg Pro Asp Leu Gln Pro Gly Tyr Glu

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340				345				350							
Gly	Trp	Gln	Ala	Leu	Asp	Pro	Thr	Pro	Gln	Glu	Lys	Ser	Glu	Gly	Thr
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Tyr	Cys	Cys	Gly	Pro	Val	Pro	Val	Arg	Ala	Ile	Lys	Glu	Gly	Asp	Leu
	370						375						380		
Ser	Thr	Lys	Tyr	Asp	Ala	Pro	Phe	Val	Phe	Ala	Glu	Val	Asn	Ala	Asp
	385						390								400
Val	Val	Asp	Trp	Ile	Gln	Gln	Asp	Asp	Gly	Ser	Val	His	Lys	Ser	Ile
					405						410				415
Asn	Arg	Ser	Leu	Ile	Val	Gly	Leu	Lys	Ile	Ser	Thr	Lys	Ser	Val	Gly
					420										430
Arg	Asp	Glu	Arg	Glu	Asp	Ile	Thr	His	Thr	Tyr	Lys	Tyr	Pro	Glu	Gly
							440								445
Ser	Ser	Glu	Glu	Arg	Glu	Ala	Phe	Thr	Arg	Ala	Asn	His	Leu	Asn	Lys
							455								460
Leu	Ala	Glu	Lys	Glu	Glu	Thr	Gly	Met	Ala	Met	Arg	Ile	Arg	Val	Gly
							470								480
Gln	Ser	Met	Asn	Met	Gly	Ser	Asp	Phe	Asp	Val	Phe	Ala	His	Ile	Thr
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Asn	Asn	Thr	Ala	Glu	Glu	Tyr	Val	Cys	Arg	Leu	Leu	Leu	Cys	Ala	Arg
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Thr	Val	Ser	Tyr	Asn	Gly	Ile	Leu	Gly	Pro	Glu	Cys	Gly	Thr	Lys	Tyr
															525
Leu	Leu	Asn	Leu	Asn	Leu	Glu	Pro	Phe	Ser	Glu	Lys	Ser	Val	Pro	Leu
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Cys	Ile	Leu	Tyr	Glu	Lys	Tyr	Arg	Asp	Cys	Leu	Thr	Glu	Ser	Asn	Leu
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Ile	Lys	Val	Arg	Ala	Leu	Leu	Val	Glu	Pro	Val	Ile	Asn	Ser	Tyr	Leu
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Leu	Ala	Glu	Arg	Asp	Leu	Tyr	Leu	Glu	Asn	Pro	Glu	Ile	Lys	Ile	Arg
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Ile	Leu	Gly	Glu	Pro	Lys	Gln	Lys	Arg	Lys	Leu	Val	Ala	Glu	Val	Ser
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Leu	Gln	Asn	Pro	Leu	Pro	Val	Ala	Leu	Glu	Gly	Cys	Thr	Phe	Thr	Val
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Glu	Gly	Ala	Gly	Leu	Thr	Glu	Glu	Gln	Lys	Thr	Val	Glu	Ile	Pro	Asp
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Pro	Val	Glu	Ala	Gly	Glu	Glu	Val	Lys	Val	Arg	Met	Asp	Leu	Leu	Pro
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Leu	His	Met	Gly	Leu	His	Lys	Leu	Val	Val	Asn	Phe	Glu	Ser	Asp	Lys
															670
Leu	Lys	Ala	Val	Lys	Gly	Phe	Arg	Asn	Val	Ile	Ile	Gly	Pro	Ala	
															685

&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 717

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

&lt;400&gt; SEQUENCE: 6

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Asn	Gly	Arg	Asp 20	His	His	Thr	Ala	Asp 25	Leu	Cys	Arg	Glu	Lys 30	Leu	Val
Val	Arg	Arg	Gly 35	Gln	Pro	Phe	Trp 40	Leu	Thr	Leu	His	Phe 45	Glu	Gly	Arg
Asn	Tyr	Glu	Ala	Ser	Val	Asp 55	Ser	Leu	Thr	Phe	Ser 60	Val	Val	Thr	Gly
Pro 65	Ala	Pro	Ser	Gln	Glu 70	Ala	Gly	Thr	Lys	Ala 75	Arg	Phe	Pro	Leu	Arg 80
Asp	Ala	Val	Glu	Glu 85	Gly	Asp	Trp	Thr	Ala 90	Thr	Val	Val	Asp	Gln 95	Gln
Asp	Cys	Thr	Leu 100	Ser	Leu	Gln	Leu	Thr 105	Thr	Pro	Ala	Asn 110	Ala	Pro	Ile
Gly	Leu	Tyr 115	Arg	Leu	Ser	Leu	Glu 120	Ala	Ser	Thr	Gly	Tyr 125	Gln	Gly	Ser
Ser	Phe 130	Val	Leu	Gly	His	Phe 135	Ile	Leu	Leu	Phe	Asn 140	Ala	Trp	Cys	Pro
Ala 145	Asp	Ala	Val	Tyr	Leu 150	Asp	Ser	Glu	Glu	Glu 155	Arg	Gln	Glu	Tyr	Val 160
Leu	Thr	Gln	Gln 165	Gly	Phe	Ile	Tyr	Gln	Gly 170	Ser	Ala	Lys	Phe	Ile 175	Lys
Asn	Ile	Pro	Trp 180	Asn	Phe	Gly	Gln	Phe 185	Glu	Asp	Gly	Ile 190	Leu	Asp	Ile
Cys	Leu	Ile 195	Leu	Leu	Asp	Val	Asn 200	Pro	Lys	Phe	Leu	Lys 205	Asn	Ala	Gly
Arg	Asp 210	Cys	Ser	Arg	Arg	Ser 215	Ser	Pro	Val	Tyr	Val 220	Gly	Arg	Val	Val
Ser 225	Gly	Met	Val	Asn	Cys 230	Asn	Asp	Asp	Gln	Gly 235	Val	Leu	Leu	Gly	Arg 240
Trp	Asp	Asn	Asn 245	Tyr	Gly	Asp	Gly	Val	Ser 250	Pro	Met	Ser	Trp	Ile 255	Gly
Ser	Val	Asp	Ile 260	Leu	Arg	Arg	Trp	Lys 265	Asn	His	Gly	Cys 270	Gln	Arg	Val
Lys	Tyr	Gly 275	Gln	Cys	Trp	Val	Phe 280	Ala	Ala	Val	Ala	Cys 285	Thr	Val	Leu
Arg	Cys 290	Leu	Gly	Ile	Pro	Thr 295	Arg	Val	Val	Thr	Asn 300	Tyr	Asn	Ser	Ala
His 305	Asp	Gln	Asn	Ser	Asn 310	Leu	Leu	Ile	Glu	Tyr 315	Phe	Arg	Asn	Glu	Phe 320
Gly	Glu	Ile	Gln 325	Gly	Asp	Lys	Ser	Glu	Met 330	Ile	Trp	Asn	Phe	His 335	Cys
Trp	Val	Glu	Ser 340	Trp	Met	Thr	Arg	Pro 345	Asp	Leu	Gln	Pro	Gly 350	Tyr	Glu
Gly	Trp	Gln 355	Ala	Leu	Asp	Pro	Thr 360	Pro	Gln	Glu	Lys	Ser 365	Glu	Gly	Thr
Tyr	Cys 370	Cys	Gly	Pro	Val	Pro 375	Val	Arg	Ala	Ile	Lys 380	Glu	Gly	Asp	Leu
Ser 385	Thr	Lys	Tyr	Asp	Ala 390	Pro	Phe	Val	Phe	Ala 395	Glu	Val	Asn	Ala	Asp 400
Val	Val	Asp	Trp 405	Ile	Gln	Gln	Asp	Asp	Gly 410	Ser	Val	His	Lys	Ser	Ile 415
Asn	Arg	Ser	Leu 420	Ile	Val	Gly	Leu	Lys 425	Ile	Ser	Thr	Lys	Ser	Val	Gly 430
Arg	Asp	Glu	Arg	Glu	Asp	Ile	Thr	His	Thr	Tyr	Lys	Tyr	Pro	Glu	Glu

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435	440	445
Ser Ser Glu Glu Arg Glu Ala Phe Thr Arg Ala Asn His Leu Asn Lys		
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Leu Ala Glu Lys Glu Glu Thr Gly Met Ala Met Arg Ile Arg Val Gly		
465	470	475
Gln Ser Met Asn Met Gly Ser Asp Phe Asp Val Phe Ala His Ile Thr		
485	490	495
Asn Asn Thr Ala Glu Glu Tyr Val Cys Arg Leu Leu Leu Cys Ala Arg		
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Thr Val Ser Tyr Asn Gly Ile Leu Gly Pro Glu Cys Gly Thr Lys Tyr		
515	520	525
Leu Leu Asn Leu Asn Leu Glu Pro Phe Ser Glu Lys Ser Val Pro Leu		
530	535	540
Cys Ile Leu Tyr Glu Lys Tyr Arg Asp Cys Leu Thr Glu Ser Asn Leu		
545	550	555
Ile Lys Val Arg Ala Leu Leu Val Glu Pro Val Ile Asn Ser Tyr Leu		
565	570	575
Leu Ala Glu Arg Asp Leu Tyr Leu Glu Asn Pro Glu Ile Lys Ile Arg		
580	585	590
Ile Leu Gly Glu Pro Lys Gln Lys Arg Lys Leu Val Ala Glu Val Ser		
595	600	605
Leu Gln Asn Pro Leu Pro Val Ala Leu Glu Gly Cys Thr Phe Thr Val		
610	615	620
Glu Gly Ala Gly Leu Thr Glu Glu Gln Lys Thr Val Glu Ile Pro Asp		
625	630	635
Pro Val Glu Ala Gly Glu Glu Val Lys Val Arg Met Asp Leu Leu Pro		
645	650	655
Leu His Met Gly Leu His Lys Leu Val Val Asn Phe Glu Ser Asp Lys		
660	665	670
Leu Lys Ala Val Lys Gly Phe Arg Asn Val Ile Ile Gly Pro Ala Gln		
675	680	685
Glu Gln Pro Phe Pro Gln Pro Glu Gln Pro Phe Gln Pro Glu Gln Pro		
690	695	700
Phe Gln Glu Gln Pro Phe Pro Gln Pro Glu Gln Pro Phe		
705	710	715

&lt;210&gt; SEQ ID NO 7

&lt;211&gt; LENGTH: 747

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

&lt;400&gt; SEQUENCE: 7

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Asn Gly Arg Asp His His Thr Ala Asp Leu Cys Arg Glu Lys Leu Val		
20	25	30
Val Arg Arg Gly Gln Pro Phe Trp Leu Thr Leu His Phe Glu Gly Arg		
35	40	45
Asn Tyr Glu Ala Ser Val Asp Ser Leu Thr Phe Ser Val Val Thr Gly		
50	55	60
Pro Ala Pro Ser Gln Glu Ala Gly Thr Lys Ala Arg Phe Pro Leu Arg		
65	70	80

Asp	Ala	Val	Glu	Glu	Gly	Asp	Trp	Thr	Ala	Thr	Val	Val	Asp	Gln	Gln
			85						90					95	
Asp	Cys	Thr	Leu	Ser	Leu	Gln	Leu	Thr	Thr	Pro	Ala	Asn	Ala	Pro	Ile
			100					105				110			
Gly	Leu	Tyr	Arg	Leu	Ser	Leu	Glu	Ala	Ser	Thr	Gly	Tyr	Gln	Gly	Ser
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Ser	Phe	Val	Leu	Gly	His	Phe	Ile	Leu	Leu	Phe	Asn	Ala	Trp	Cys	Pro
			130			135					140				
Ala	Asp	Ala	Val	Tyr	Leu	Asp	Ser	Glu	Glu	Glu	Arg	Gln	Glu	Tyr	Val
					150					155					160
Leu	Thr	Gln	Gln	Gly	Phe	Ile	Tyr	Gln	Gly	Ser	Ala	Lys	Phe	Ile	Lys
				165					170					175	
Asn	Ile	Pro	Trp	Asn	Phe	Gly	Gln	Phe	Glu	Asp	Gly	Ile	Leu	Asp	Ile
			180					185					190		
Cys	Leu	Ile	Leu	Leu	Asp	Val	Asn	Pro	Lys	Phe	Leu	Lys	Asn	Ala	Gly
			195				200					205			
Arg	Asp	Cys	Ser	Arg	Arg	Ser	Ser	Pro	Val	Tyr	Val	Gly	Arg	Val	Val
			210			215					220				
Ser	Gly	Met	Val	Asn	Cys	Asn	Asp	Asp	Gln	Gly	Val	Leu	Leu	Gly	Arg
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Trp	Asp	Asn	Asn	Tyr	Gly	Asp	Gly	Val	Ser	Pro	Met	Ser	Trp	Ile	Gly
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Ser	Val	Asp	Ile	Leu	Arg	Arg	Trp	Lys	Asn	His	Gly	Cys	Gln	Arg	Val
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Lys	Tyr	Gly	Gln	Cys	Trp	Val	Phe	Ala	Ala	Val	Ala	Cys	Thr	Val	Leu
			275				280					285			
Arg	Cys	Leu	Gly	Ile	Pro	Thr	Arg	Val	Val	Thr	Asn	Tyr	Asn	Ser	Ala
			290			295					300				
His	Asp	Gln	Asn	Ser	Asn	Leu	Leu	Ile	Glu	Tyr	Phe	Arg	Asn	Glu	Phe
				310					315					320	
Gly	Glu	Ile	Gln	Gly	Asp	Lys	Ser	Glu	Met	Ile	Trp	Asn	Phe	His	Cys
				325				330						335	
Trp	Val	Glu	Ser	Trp	Met	Thr	Arg	Pro	Asp	Leu	Gln	Pro	Gly	Tyr	Glu
			340					345				350			
Gly	Trp	Gln	Ala	Leu	Asp	Pro	Thr	Pro	Gln	Glu	Lys	Ser	Glu	Gly	Thr
			355				360					365			
Tyr	Cys	Cys	Gly	Pro	Val	Pro	Val	Arg	Ala	Ile	Lys	Glu	Gly	Asp	Leu
			370			375					380				
Ser	Thr	Lys	Tyr	Asp	Ala	Pro	Phe	Val	Phe	Ala	Glu	Val	Asn	Ala	Asp
				390					395					400	
Val	Val	Asp	Trp	Ile	Gln	Gln	Asp	Asp	Gly	Ser	Val	His	Lys	Ser	Ile
			405					410						415	
Asn	Arg	Ser	Leu	Ile	Val	Gly	Leu	Lys	Ile	Ser	Thr	Lys	Ser	Val	Gly
			420					425					430		
Arg	Asp	Glu	Arg	Glu	Asp	Ile	Thr	His	Thr	Tyr	Lys	Tyr	Pro	Glu	Gly
			435				440								



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500	505	510
Thr Val Ser Tyr Asn Gly Ile	Leu Gly Pro Glu Cys Gly Thr Lys Tyr	
515	520	525
Leu Leu Asn Leu Asn Leu Glu	Pro Phe Ser Glu Lys Ser Val Pro Leu	
530	535	540
Cys Ile Leu Tyr Glu Lys Tyr	Arg Asp Cys Leu Thr Glu Ser Asn Leu	
545	550	555
Ile Lys Val Arg Ala Leu Leu	Val Glu Pro Val Ile Asn Ser Tyr Leu	
565	570	575
Leu Ala Glu Arg Asp Leu Tyr	Leu Glu Asn Pro Glu Ile Lys Ile Arg	
580	585	590
Ile Leu Gly Glu Pro Lys Gln	Lys Arg Lys Leu Val Ala Glu Val Ser	
595	600	605
Leu Gln Asn Pro Leu Pro Val	Ala Leu Glu Gly Cys Thr Phe Thr Val	
610	615	620
Glu Gly Ala Gly Leu Thr Glu	Glu Gln Lys Thr Val Glu Ile Pro Asp	
625	630	635
Pro Val Glu Ala Gly Glu Glu	Val Lys Val Arg Met Asp Leu Leu Pro	
645	650	655
Leu His Met Gly Leu His Lys	Leu Val Val Asn Phe Glu Ser Asp Lys	
660	665	670
Leu Lys Ala Val Lys Gly Phe	Arg Asn Val Ile Ile Gly Pro Ala Gln	
675	680	685
Glu Gln Pro Phe Pro Gln Pro	Glu Gln Pro Phe Gln Pro Glu Gln Pro	
690	695	700
Phe Gln Glu Gln Pro Phe Pro	Gln Pro Glu Gln Pro Phe Gln Glu Gln	
705	710	715
Pro Phe Pro Gln Pro Glu Gln	Pro Phe Gln Pro Glu Gln Pro Phe Gln	
725	730	735
Glu Gln Pro Phe Pro Gln Pro	Glu Gln Pro Phe	
740	745	
<210> SEQ ID NO 8		
<211> LENGTH: 6		
<212> TYPE: PRT		
<213> ORGANISM: Artificial Sequence		
<220> FEATURE:		
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic		
6xHis tag		
<400> SEQUENCE: 8		
His His His His His His		
1 5		

The invention claimed is:

1. A peptide comprising the amino acid sequence of SEQ ID NO: 1.
2. The peptide of claim 1, characterized in that the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.
3. The peptide of claim 1, characterized in that the peptide comprises additional amino acids, one or more linkers and/or one or more functional groups.
4. The peptide of claim 1, characterized in that the peptide additionally comprises a sequence of at least 25 consecutive amino acids from SEQ ID NO: 5.
5. The peptide of claim 1, characterized in that the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6 and SEQ ID NO: 7.
6. A method for the in vitro detection of antibodies against the peptide of claim 1 in a sample, comprising the steps of:
  - i) contacting a sample with the peptide of claim 1 in vitro, and
  - ii) detecting binding of antibodies in the sample to the peptide, thereby detecting the antibodies.
7. The method of claim 6, characterized in that the sample is a body fluid or is obtained from a body fluid.
8. The method of claim 6, characterized in that the peptide is immobilized on a support.
9. The method of claim 6, characterized in that the method is carried out in an ELISA format.

**25**

**10.** A kit for performing the method of claim **6**, said kit comprising the peptide of claim **1** immobilized on a support.

**11.** The kit of claim **10**, characterized in that the kit is an ELISA or strip test.

**12.** A nucleic acid comprising a nucleic acid sequence 5 encoding SEQ ID NO:1.

**13.** A transformed microorganism transformed with a vector comprising the nucleic acid of claim **12**, or a transfected cell transfected with a vector comprising the nucleic acid of claim **12**.

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**26**